ISSN (print) 0093-4666 © 2012. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/121.435

Volume 121, pp. 435–445 *July–September 2012*

Type studies on four *Entoloma* species from South China

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Abstract — The types of *Entoloma caespitosum*, *E. carneobrunneum*, *E. metuloideum*, and *E. pseudogriseoalbum* from South China were studied. *Entoloma carneobrunneum* and *E. caespitosum* are shown to be conspecific, *E. pseudogriseoalbum* is revised based on re-examination of the holotype and additional material, and *E. metuloideum* is referred to *Inocybe* but requires further study to establish its relationships with other species in that genus.

Key words — morphology, revision, taxonomy

Introduction

Entoloma (Fr.) P. Kumm. is very species-rich with over 1000 species reported worldwide (Kirk et al. 2008), yet only 22 species have been described originally from China (Bi et al. 1986, He et al. 2011, 2012, Li & Li 2009, Li et al. 2009, Ying 1995, Zhang & Li 2002a,b, Zhang et al. 1994a,b). Few additional records have been added since their publication, probably because some taxa were described poorly or diagnosed incorrectly. During our recent study of the holotypes and additional material, we discovered that essential taxonomic characters of some type specimens differ considerably from the original published descriptions. Here we describe amended concepts based on our thorough reexamination of four species from South China.

Materials & methods

Morphological descriptions

Type specimens and additional collections of *Entoloma caespitosum*, *E. carneobrunneum*, *E. metuloideum*, and *E. pseudogriseoalbum* were studied. All collections cited are deposited at the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM).

Original Latin descriptions are copied from Zhang et al. (1994a) and Bi et al. (1986), and redescriptions are compiled from the re-examinations by the present authors. Macro-morphological descriptions are based on field notes and examination of voucher specimens. Micro-morphological data were obtained from dried material observed under a light microscope. Basidiospores, basidia, cystidia, and pileipellis were observed in 5% KOH or 1% Congo Red, while KOH-soluble pigments were examined in distilled H_2O. All measurements were made in 5% KOH. In basidiospore descriptions, Q = the length/width ratio of a basidiospore in profile view. Basidiospore dimensions exclude the hilar appendix or apiculus in profile view. Herbarium codes follow Holmgren et al. (1990).

Molecular procedures and phylogenetic analysis

Genomic DNA was extracted from dried specimens by using a modified CTAB procedure of Doyle & Doyle (1987). ITS rDNA was amplified using primers ITS4 and ITS5 (White et al. 1990; Gardes & Bruns 1993). Amplification was performed in 20 µl volumes containing 0.5 µl template DNA, 8.5 µl distilled water, 0.5 µl of each primer and 10 µl PCR mix. The reactions were carried out with 35 cycles by the following conditions: denaturation (95°C, 30 s), annealing (52°C, 30 s), extension (72°C, 1 min), final extension (72°C, 10 min).

The primers used for sequencing were the same as those for amplification. Sequences generated in this study were deposited in GenBank (Table 1).

Table 1. Specimens included in the molecular analysis.

Species	Specimen No.	Geographic origin	Sequence No.
Entoloma abortivum	GDGM 27313	China: Jilin, Changbai Mountain	JQ291565
	HMJAU 1955	China: Jilin, Changbai Mountain	JQ281483
E. caespitosum	GDGM 27564	China: Hainan, Jianfengling National Nature Reserve	JQ281477
[as *E. carneobrunneum*]	GDGM 24026	China: Guangdong, Yangchun	JQ281491
[as *E. carneobrunneum*]	GDGM 24025	China: Guangdong, Yangchun	JQ281490
E. clypeatum	GDGM 28830	China: Beijing, Huairou	JQ281479
E. conferendum	HKAS 48953	China: Sichuan, Daocheng	JQ281484
E. flavidum	GDGM 24473	China: Guangdong, Shenzhen	JQ281481
E. incanum	HKAS 54614	China: Yunnan, Jingdong Ainaoshan	JQ281488
E. luteum	GDGM 27698	China: Hainan, Jianfengling National Nature Reserve	JQ281486
E. mastoideum	GDGM 28820	China: Guangdong, Yangchun	JQ281476
	GDGM 26597	China: Guangdong, Yangchun	JQ291564
E. omiense	GDGM 27563	China: Hainan, Jianfengling National Nature Reserve	JQ281487
	GDGM 27229	China: Jiangxi, Fuzhou	JQ291566
E. petchii	HKAS 56716	China: Yunnan, Baoshan	JQ281485
E. praegracile	GDGM 29251	China: Guangdong, Nanling National Forest Park	JQ281482
*E. sepium**	—	Italy	JF908001
E. stylophorum	GDGM 25736	China: Hainan, Jianfengling National Nature Reserve	JQ281480
*E. versatile**	UBC F16567	Canada	FJ627026
Inocephalus sp.*	—	Argentina	DQ490636
Clitopilus crispus	GDGM 29931	China: Guangdong, Guangzhou	JQ281489
C. prunulus	HKAS 45904	China: Yunnan, Tengchong	JQ281478

* Sequence downloaded from GenBank

Maximum parsimony (MP) was determined using PAUP* version 4.0b10 (Swofford 2003) for phylogenetic analyses. DNA sequences were edited and aligned with Bioedit and manually modified where necessary. *Clitopilus prunulus* and *C. crispus* were included as outgroup. All characters were treated as unordered and equal weighted, gaps were treated as missing data, and bootstrap values were calculated from 1000 replicates.

Taxonomy

Entoloma caespitosum W.M. Zhang, Acta Mycol. Sin. 13: 192, 1994. FIGS 1–2

≡ *Entoloma carneobrunneum* W.M. Zhang, Acta Mycol. Sin. 13: 193, 1994.

Original Latin description of *Entoloma caespitosum*

Pileus 3–5 cm latus, purpureo-ruber vel rubro-brunneus, conicus, convexus vel planus, in centro acuti umbonatus, glaber, carnosus, margine integris et estriato. Contextus pallide purpureo-ruber, ad stipitem circit. 0.5–1 mm, inodorus. Lamellae primo albae, demum pallide roseolae, subconfertae adnatae vel adnexae, inaequales, marginibus integris. Stipes centralis, cylindricus vel clavatus, 3–9 cm longus, 2–6 mm crassus, albus vel subalbus, fistulosus, fragilis, ad basin mycelio albo. Sporae 6–8-angulosae, apiculatae, 7.5–11.5 (–12.8) × 5–7.5 µm, laeves, pallide roseolae, inamyloideae. Basidia clavata, 30–40 × 7–10 µm, 2-spora, hyalina, sterigmatibus 4–6.5 µm longis. Pleurocystidia clavata, ad apicem acuta, 35–46 × 8–10 µm, hyalina, rara. Cheilocystidia cylindrica, 30–38 × 4–5 µm, hyalina, gregaria, numerosa. Trama hymenophoralis parallelis. Eicute [sic] pilei ex hyphis repentibus, hyalinis. Hyphae defibulatae.

Original Latin description of Entoloma carneobrunneum

Pileus 2–4 cm latus, atro-carneo-brunneus, in centro atrobrunneus, siccus, plano-umboatus, glaber, carnosus. Contextus albus, 0.5–1.5 mm crassus, inodorus. Lamellae pallide rosaceae vel carneo-albae, late adnatae vel breviter decurrentes, subdistantes, inaequales, marginibus integris. Stipes centralis, cylindricus vel subclavatus, 2–4 cm longus, 3–5 mm crassus, albus, fistulosus, fibrus, glaber. Sporae (5–)6–7-angulosae, saepe 6-angulosae, apiculatae, 7.7–10 × 5–7.7 µm, laeves, pallide roseolae, inamyloideae, 1–2-guttulatae. Basidia clavata, 26–36 × 9–10 µm, 4-spora, hyalina, sterigmatibus 2.6–3.8 µm longis. Pleurocystidia desunt. Cheilocystidia cylindrica, nonnumquam ad apicem capitata, 40–90 × 4–7 µm, hyalina, numerosa. Trama hymenophoralis parallelis. Epicute pilei ex hyphis repentibus, cylindricis, hyalinis. Hyphae defibulatis.

Revised description

BASIDIOMATA medium-sized. PILEUS 2–5 cm broad, conical to convex at first, expanding to plano-convex, applanate or upturned with a very pronounced, papillate umbo, slightly to distinctly striate almost up to center, dry, not hygrophanous, smooth, with straight and entire to crenulate margin sometimes exceeding gills, pale brown to brown at the umbo, paler elsewhere, carneous white (4B2) to carneous brown (7C3). LAMELLAE adnate-emarginate to adnexed, subventricose, up to 4 mm broad, white at first becoming pinkish with age, moderately crowded, thin, with concolorous and irregular edge, with 3–4 tiers of lamellulae. STIPE central, 30–90 × 2–6 mm, cylindrical, equal, smooth, slightly polished, surface dry, concolorous with or slightly paler than pileus, hollow, brittle, with white tomentum at base. CONTEXT white, thin. ODOR not distinctive, TASTE not known.

Fig. 1. *Entoloma caespitosum* basidiomata.
a. GDGM 24026. b. GDGM 27564.c. GDGM 24025.

Basidiospores 8.5–10.5 × 6–7.5 μm, Q = 1.23–1.54, heterodiametric, relatively thin-walled, 6–9-angled with blunt angles in profile view. Basidia 30–45 × 8–12 μm, clavate, 4-spored, sometimes 2-spored. Lamellar edge sterile. Cheilocystidia in clusters along the lamellar margin, 30–80 × 5–11 μm, cylindrical to narrowly clavate, hyaline, thin-walled. Pleurocystidia absent. Hymenophoral trama parallel, made up of cylindrical elements, 50–110 × 5–16 μm. Pileipellis a cutis, made up of repent hyphae, terminal cells cylindrical to subclavate, 5–12

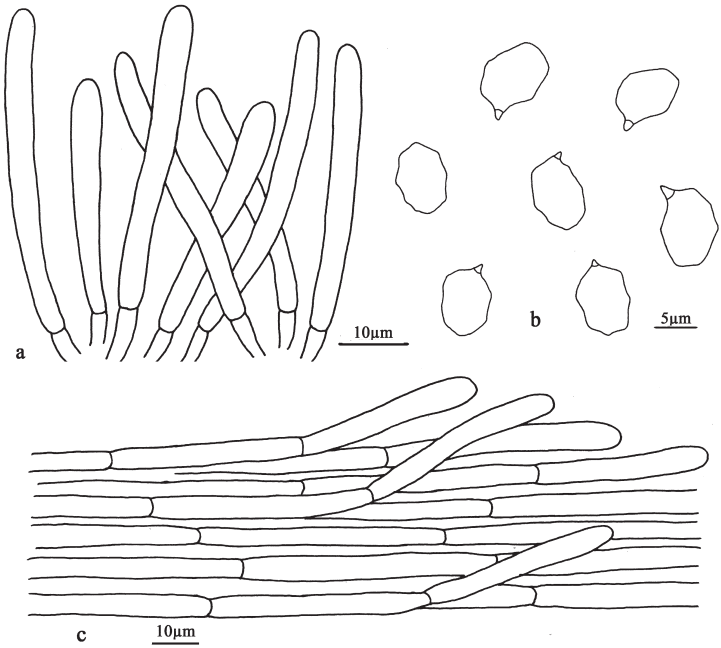

Fig. 2. *Entoloma caespitosum* (GDGM 16476), microscopic structures.
a. Cheilocystidia. b. Basidiospores. c. Pileipellis.

μm wide, with pale yellow brownish, intracellular pigment. Pileitrama regular, consisting of cylindrical hyphae. Stipitipellis a cutis of cylindrical to subclavate hyphae, 3–9 μm in diameter. Brilliant granules very abundant in lamellar trama and pileitrama. Oleiferous hyphae abundant in lamellar trama. Clamp connections absent in all tissues.

Habitat: Scattered to caespitose, on sandy soil of tropical rainforest with *Ficus*, *Castanopsis*, *Machilus*, *Cyclobalanopsis*, and *Taxodiaceae*.

Specimens examined — **China. Hainan Province**: Ledong County, Jianfengling National Forest Park, at 18°36′–52′N 108°40′–109°02′E, alt. 680–730 m, 21 October 1988, Chen Huan-Qiang (GDGM 16476, holotype); 21 August 1988, Chen Huan-Qiang (GDGM 15619); 28 July 2009, Li Tai-Hui & Huang Hao (GDGM 27564); 850–900 m, 11 June 1990, Chen Huan-Qiang (GDGM 16726, holotype of *E. carneobrunneum*); **Guangdong Province**: Zhaoqing, Dinghushan, 21 November 1992, Li Tai-Hui (GDGM 18333, as *E. carneobrunneum*); Yangchun County, Ehuangzhang, Xianjiatong, 26 April 2005, Li Tai-Hui & Wu Li-Min (GDGM 24136, as *E. carneobrunneum*); 26 April 2005, Li Tai-Hui & Wu Li-Min (GDGM 24025, as *E. carneobrunneum*); 26 April 2005, Li Tai-Hui & Wu Li-Min (GDGM 24026, as *E. carneobrunneum*).

Commentary — The caespitose habit, plano-convex or applanate pileus with papillate umbo, 6–9-angled basidiospores with blunt angles, and the abundant cheilocystidia are distinctive for *E. caespitosum*.

The holotype consists of caespitose basidiocarps in good condition, and another collection (GDGM 15619) in GDGM was also cited by Zhang et al. (1994a) in the protologue of *E. caespitosum*. Re-examination of the voucher collections showed that — contrary to the original description — the pileus is umbonate with a striate margin, most basidia have four slender sterigmata, and apically acute clavate pleurocystidia are absent from the hymenium.

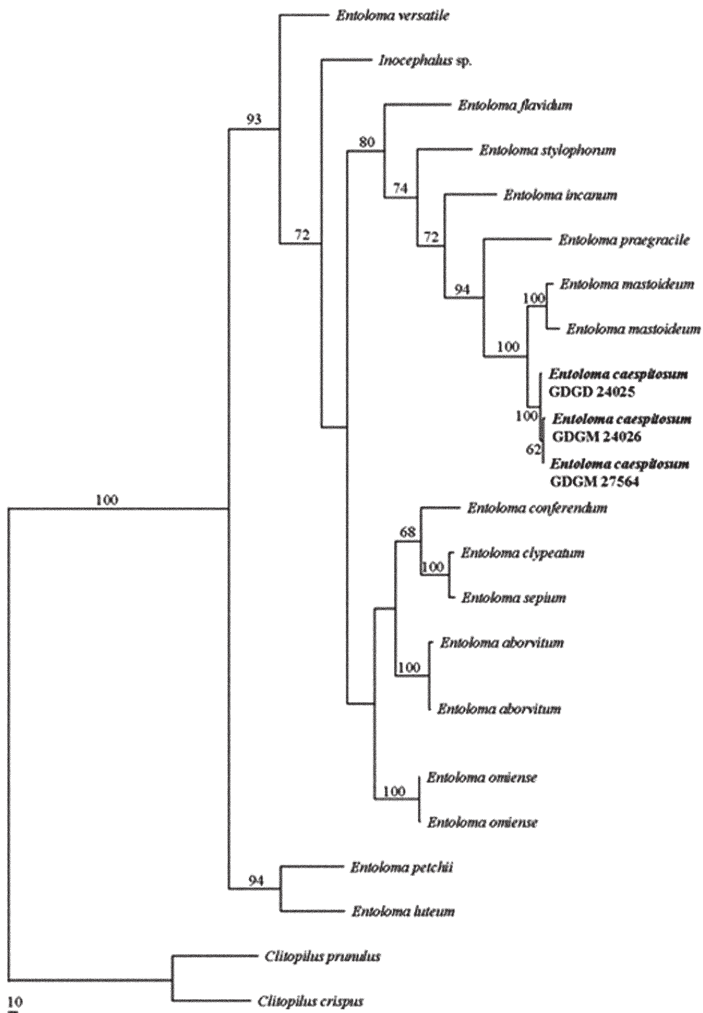

Fig. 3. Phylogeny of *Entoloma caespitosum* and related species, generated by Maximum Parsimony analysis based on ITS sequences. Values above the branches are parsimony bootstraps >50%.

In the same publication, Zhang et al. (1994a) described a later collection from the same area (GDGM 16726) as *E. carneobrunneum*, said to differ from *E. caespitosum* mainly by the absence of pleurocystidia. However, we found no differences between the two holotypes in macro- and microscopic characters. Comparisons of the *E. caespitosum* specimens and four *E. carneobrunneum* collections (GDGM 18333, GDGM 24136, GDGM 24025, and GDGM 24026) revealed no convincing morphological or ITS sequence (Fig. 3) differences between the two taxa. Therefore, we consider *E. carneobrunneum* a synonym of *E. caespitosum*.

A 2009 collection (GDGM 27564) from the *E. caespitosum* type locality provided additional information for this taxon (Fig. 1). It has mostly the same characters (caespitose habit, plano-convex pileus with distinct papillate umbo, thin-walled basidiospores with 6–9 angles in profile view, abundant cheilocystidia forming a sterile edge) as cited in the protologue of *E. caespitosum*, but has a carneous white pileus in the field as shown in the photographs (Fig. 1). As it otherwise shares the same diagnostic characters with *E. caespitosum*, we revise the pileus color to carneous white to carneous brown based on the holotype and the new material. In addition, GDGM 27564, GDGM 24025 and GDGM 24026 cluster together phylogenetically with high statistical support based on rDNA ITS sequence data (Fig. 3). Unfortunately, no DNA sequences were successfully generated from the holotype.

Another scattered to caespitose species, *E. mastoideum* T.H. Li & Xiao Lan He, described recently from South China, shares with *E. caespitosum* a plano-convex to applanate pileus with papillate umbo (He et al. 2011). However, *E. mastoideum* has larger basidiospores (9.5–12(–13) × 7–8(–8.8) µm). Furthermore, the molecular data indicated that the two species are quite different from each other (Fig. 3).

Entoloma metuloideum W.M. Zhang & T.H. Li, Mycosystema 21: 153, 2002. Fig. 4

Original Latin description

Pileus 6.5 cm latus, plano-umbonatus carnosus, pallide purpureo-vitellinus, flavo-brunneus vel atro-brunneus in centro, siccus, humidus, glaber. Contextus flavidus, ad stipitem circit 2 mm, indorus, sapor mitis. Lamellae pallide viridi-flavae, subdistantes, inaequales, marginibus integris, adnate vel breviter decurrentes. Stipes centralis, 6.5 cm longus, 6 mm crassus, vitellinus, cylindricus, ad apicem tenuis, in basi inflatus, villosus, fibrus, solidus. Sporae 6–9.6 × 4.8–7.2 µm, 4–5(–6)-angulosae, polymorphae, pallide roseolae, glabrae, apiculatae. Basidia 19–26 × 6–8 µm, clavata, 4-spora, hyalina, sterigmatibus 2.4 µm longis. Pleurocystidia (metuloidae) 38–62 × 12–17 µm, clavata vel subfusiformia, hyalina, numerosa, apicibus appendicibus granulatis vel globosis praeditis, membranis crassis. Cheilocystidia similia pleurocystidiis (metuloidis) crassitunicatis vel tenuitunicata, 19–24 × 5–7 µm, cylindrica vel subclavata, hyalina, caepitosa [sic], numerosa. Trama hymenophori subparallela, hyphis inflatis et cystoformibus tenuitunicatis hyalinis praedita. Pileipellis ex hyphis 4.8–7.2 µm crassis, repentibus, cylindricis, hyalinis composita. Fibulae praesentes.

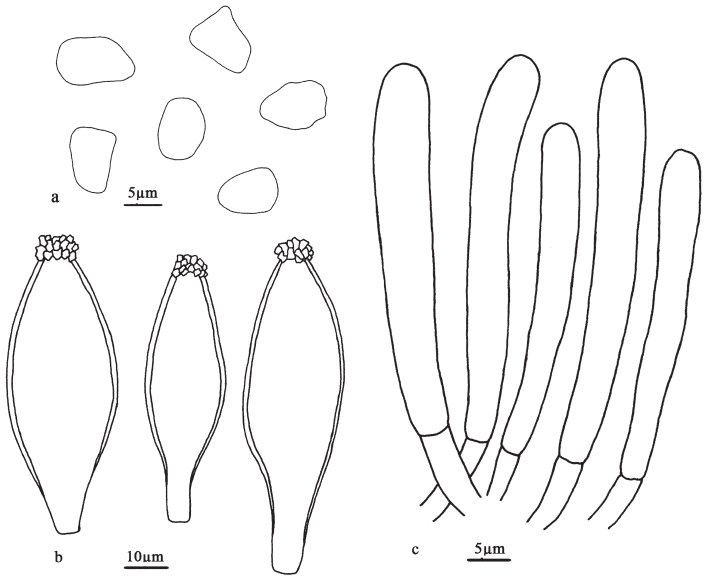

Fig. 4. *Entoloma metuloideum* [= an *Inocybe*] (GDGM 18773), microscopic structures. a. Basidiospores. b. Pleurocystidia. c. Cheilocystidia.

Revised description

Basidioma medium-sized. Pileus to 6.5 cm broad, applanate, umbonate, pale yellowish brown with purple tinge, yellow-brown to brown at the umbo, dry, hygrophanous, glabrous, faintly striate at margin. Lamellae adnate with short decurrent tooth, greenish yellow, moderately distant, moderately thick, up to 8 mm broad, with concolorous and uneven edge, with 2 tiers of lamellulae. Stipe central, 65 × 6 mm, dark yellow with reddish tinge, cylindrical, gradually enlarged towards the inflated base, dry, solid, fibrillose. Context yellowish, 2 mm thick at disk. Odor and taste not distinctive.

Basidiospores 7.5–10 × 6–7.5 μm, 4–5(–6)-angled, polymorphic, without an apiculus, not truly angular, smooth, yellowish brown in KOH. Basidia 19–26 × 6–8 μm, clamped at base, clavate, very pale yellow brown in KOH, 4-spored, sterigmata 2.4 μm in length. Lamellar trama composed of thin-walled hyphae 5–14 μm in width, pale yellow brownish in KOH. Pleurocystidia 58–100 × 15–27 μm, cell walls mostly 0.5–2.5 μm thick, fusiform-ventricose with a short pedicel, colorless, apices obtuse and usually crystalliferous. Cheilocystidia of two types: (1) metuloids similar to pleurocystidia; (2) cylindrical to narrowly clavate, 19–24 × 5–7 μm, in clusters, hyaline, thin-walled. Pileipellis a cutis, consisting of cylindrical hyphae, terminal elements 5–10 μm in diam, cylindrical to clavate. Clamp connections present in pileipellis.

Habitat: Solitary, on soil in deciduous woods.

Specimen examined — **China. Guangdong Province:** Tianjingshan, Nanling National Nature Reserve, alt. 1700 m, 26 October 1995, Zhang Wei-Min (GDGM 18773, holotype).

Commentary — The holotype is in good condition and consists of a robust yellow-brown basidioma provided with field notes. Re-examination of this holotype showed that the pale brown basidiospores are not truly angular as in *Entoloma* species and lack the distinctive prominent apiculus typical of *Entoloma* taxa. The combined features of brownish green lamellae, yellow-brown and not truly angular basidiospores, pale yellow-brown lamellar trama, and distinctive thick-walled metuloid pleurocystidia indicate that this taxon represents *Inocybe* (Fr.) Fr.. Further comparative study is required to establish the specimen's relationship with other *Inocybe* species.

Entoloma pseudogriseoalbum Z.S. Bi, Acta Mycol. Sin. 5: 164, 1986. Fig. 5

Original Latin description

Pileus 6–14 mm latus, primo campanulatus vel convexus, dein planus in centro subumbilicus, siccus, albus vel pallide brunneus, carnosus, in centro tomentosus vel glaber, ad marginem radiate striatus. Contexto albo, tenui, sapor mitis. Lamellae primo albae, demum rosaceae, subdistantes, brever decurrentes, inaequalis. Stipe centralis, albus, 2–4 cm longus, 2–3 mm crassus, cylindricus, fistulosus, fibrus, incrassatus basin versus. Sporae quadrate, 12–13 × 10–12 µm, pallide rosaceae. Basidia 50–60 × 10–12 µm, clavata, 4-spora, subhyalina. Pleurocystidia et cheilocystidia desunt. Trama hymenophoralis parallelis. Epicute pilei ex hyphis evertibus, subhyalinis. Hyphis fibulatis.

Revised description

Basidiomata small. Pileus 6–14 cm broad, convex to campanulate at first, expanding to plano-convex or applanate, with a slightly depressed or subumbilicate center, sometimes with a small and indistinct papillae in the depression, smooth, tomentulose to minutely squamulose in the center, whitish when young becoming pale brownish, radially striate almost to center, dry, not hygrophanous, with straight and entire margin. Lamellae subdecurrent, moderately distant, ≤2 mm broad, white at first becoming pinkish with age, thin, with concolorous and irregular edge, with 2 tiers of lamellulae. Stipe central, 20–50 × 1–2 mm, cylindrical, equal, hollow, innately fibrillose, dry, with white tomentum at base. Context white, thin. Odor not distinctive, taste mild.

Basidiospores 7.0–9.0 µm, cuboid or quadrate in profile view, relatively thin-walled. Basidia 34–47 × 10–12 µm, clavate, 4-spored, with sterigmata of 4–5 µm in length, without clamp-connections at base. Lamellar edge sterile. Cheilocystidia in clusters along the lamellar margin, cylindrical to narrowly clavate, 50–110 × 6–10 µm, hyaline, thin-walled. Pleurocystidia absent. Hymenophoral trama parallel, made up of cylindrical elements. Pileipellis a cutis with a transition to a trichoderm, terminal cells cylindrical to subclavate, 7–12 µm wide, with pale yellow brownish, intracellular pigment.

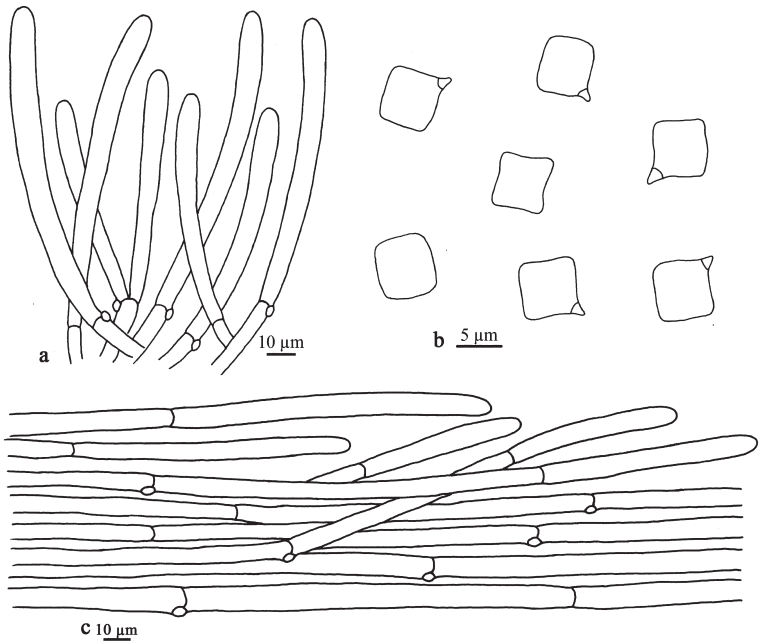

Fig. 5. *Entoloma pseudogriseoalbum* (GDGM 7457), microscopic structures.
a. Cheilocystidia. b. Basidiospores. c. Pileipellis.

Pileitrama regular of cylindrical hyphae. Stipitipellis a cutis of narrow and cylindric hyphae, 3–9 μm in diameter. Caulocystidia absent. Brilliant granules absent. Oleiferous hyphae rare. Clamp connections present in all tissues.

Habitat: Scattered or solitary, on soil in deciduous woods.

Specimens examined — **China. Guangdong Province:** Yangshan County, 1 September 1984, Li Tai-Hui & Zheng Guo-Yang (GDGM 7457, holotype); Heyuan, 13 September 2001, Li Tai-Hui (GDGM 20485); Yangchun County, 18 August 2004, Li Tai-Hui (GDGM 21738).

Commentary — *Entoloma pseudogriseoalbum* is characterized by the umbilicate pileus, cuboid basidiospores and abundant cylindrical cheilocystidia.

In the original protologue, the basidiospores were described as 12–13 × 10–12 μm, and cheilocystidia were absent (Bi et al. 1986). However, the basidiospores of the type collection are less than 10 μm in size, and the cheilocystidia are abundant, forming a sterile band on the lamellar edge. Two collections collected from Heyuan (GDGM 20485) and Yangchun (GDGM 21738) not far from the type locality are identical with the holotype of *E. pseudogriseoalbum*.

Entoloma talisporum Corner & E. Horak, originally described from Papua New Guinea, resembles *E. pseudogriseoalbum* in its small basidiomata, subumbilicate whitish pileus, subdecurrent lamellae, and cuboid basidiospores, but differs in its smaller basidiospores (6–8 μm) and absence of cheilocystidia (Horak 1976).

Acknowledgements

The authors express sincere gratitude to Dr. Timothy J. Baroni (State University of New York – College at Cortland) and Dr. Genevieve M. Gates (University of Tasmania), who reviewed the manuscript. The research was financed by the National Natural Science Foundation of China (Project Nos. 30499340, 30970023 and 31070024).

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